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Abstract**Organic-Inorganic Hybrid Mesoporous Silica Materials and their Application as
Matrix for Protein Molecules**

Hua Dong
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Transparent, organic-inorganic hybrid mesoporous silica materials have been prepared successfully via the acid-catalyzed hydrolysis and cocondensation of tetramethyl orthosilicate and various organosiloxanes via the nonsurfactant template-directed sol-gel process. The organic groups were attached to the silica matrix via the non-hydrolyzable Si-C covalent bond and functioned as network modifier. The synthesis conditions have been systematically studied and optimized. Nitrogen adsorption-desorption and transmission electronic microscopy characterizations show that the sol-gel materials obtained after removing the templates possess a three-dimensional network of interconnected mesopores. The pore parameters are tunable to some extent by varying the template content.

Several enzyme systems, such as horseradish peroxidase (HRP), glucose oxidase (GOx), lipase, alcohol dehydrogenase (ADH) etc. have been immobilized *in situ* in the hybrid mesostructured, or more generally, nanostructured silica matrix. The catalytic activity of immobilized enzymes has been assayed and correlated with the microstructures of the host silica materials under varied conditions. The enzymes encapsulated in the nonsurfactant-templated mesoporous sol-gel materials exhibit

remarkably higher apparent catalytic activity, from a few-fold to three-orders of magnitude greater, than those in the non-templated conventional microporous host synthesized in the absence of the templates under otherwise identical conditions

Page 21

immobilization of more than one enzyme in the mesoporous host materials has been achieved, in which one enzyme's product is another's substrate. Protein unfolding and refolding in the nanoporous host have been investigated. Thermal stability of enzymes were found to improve remarkably in the host materials.

Nonionic poly(ethylene oxide) copolymer surfactants were also utilized as templates for *in situ* immobilization of multiple enzymes. To minimize the denaturation of the enzyme in methanol which is liberated during the hydrolysis, a low-shrinkage sol-gel process is also adapted to the direct immobilization of organophosphorus anhydrolase (OPAA). The thus immobilized OPAA showed high resistance to organic solvent and is promising as the enzyme-based decontaminant. This study demonstrates that the novel sol-gel immobilization methods are versatile in terms of enzymes, templates and matrix chemical compositions, leaving much room for further modification and optimization. The optically transparent, biologically doped sol-gel mesoporous materials have potential applications in biocatalyst, biosensor devices, etc.

Preface

Since the discovery of highly ordered M41S family mesoporous materials in 1992, considerable attention has been focused on tailoring the chemical composition of these materials via the surfactant templated hydrothermal synthesis. Prior to this work, a novel nonsurfactant-templating pathway has been developed in our lab. Extraction of the template via water/solvent-extraction yields mesoporous silica materials with high surface area and pore volume as well as narrowly distributed pores of 2-6 nm. T

parameters can be tuned simply by varying the concentration of the templating compounds such as D-fructose, D-glucose, dibenzoyl-tartaric acid, malto cyclodextrin, oligopeptides, agar, etc. The ambient synthesis condition and biocompatibility of the template make direct immobilization of several enzymes practical.

The objectives of this thesis study are to extend this nonsurfactant-templating pathway to the synthesis of optically transparent, hybrid organic-inorganic silica mesoporous materials and direct immobilization of biomolecules in such materials to obtain a better understanding of the structure-property relationship, the activity of immobilized enzymes is correlated with the pore sizes and functionalities of the silica matrix. In Chapter 1, a brief review is given on the mesoporous materials and their application as the host matrix for the biomolecules immobilization. In Chapter 2, Chapter 3, a variety of organic-modified mesoporous silica materials based on the sol-gel condensation of tetramethyl orthosilicate (TMOS) and organosiloxanes are described. The synthesis of hybrid mesoporous materials with controllable pore size and pore

volume provides us with the means for direct immobilization of enzymes and of

biologically active species. In Chapter 4, we successfully applied this nonsurface templated synthesis to the immobilization of horseradish peroxidase (HRP) in the modified hybrid silica materials. The activities of the HRP encapsulated in the mesoporous hybrid hosts are significantly higher than those in the non-templated microporous materials synthesized by the conventional sol-gel process. The thermal stability is also greatly enhanced because of the confinement in the nanometer-sized cages. In Chapter 5, a multiple enzyme system, glucose oxidase and horseradish peroxidase, is co-immobilized in the pure and phenyl-modified mesoporous silica. A nonionic PEO surfactant is used as template under room temperature. The co-immobilized enzymes showed enhanced thermal stability and fast response to the glucose substrate within few seconds.

In Chapter 6, organophosphorus acid anhydrolase (OPAA), an enzyme that catalyzes the hydrolysis of the toxic organophosphate, is immobilized in the mesoporous silica matrix to develop an enzyme-based decontaminant in chemical warfare. A low-shrinking sol-gel process is employed to minimize the denaturation of the enzyme. Methanol liberated during the sol-gel process. The immobilized OPAA has a high remaining activity and resistance to organic solvents than free OPAA in solution. This novel templated immobilization is versatile in terms of enzymes, templates, and chemical compositions of the silica matrix. The optically transparent, bioactive materials have numerous potential applications as biocatalyst and biosensor for target molecules.